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Structural and Functional Analysis of the HIV gp41 Containing an Ile573 to Thr Substitution: Implications for Membrane Fusion

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Beamline(s): X25, X12B

Introduction: The envelope glycoprotein of human immunodeficiency virus type-1 (HIV-1) consists of the surface subunit gp120 and the transmembrane subunit gp41. Binding of gp120 to target cell receptors induces a conformational change in gp41, which then mediates the fusion of viral and cellular membranes. A buried isoleucine (Ile 573) in a central trimmeric coiled coil within the fusion-active gp41 ectodomain core is thought to favor this conformational activation. The role of Ile 573 in determining the structure and function of the gp120/gp41 complex was investigated by mutating this residue to threonine, a nonconservative substitution in HIV-1 that occurs naturally in simian immunodeficiency virus (SIV).

Methods and Materials: The I573T/N36(L6)C34 mutant was crystallized by the hanging-drop vapor diffusion method at room temperature. Crystals of I573T/N36(L6)C34 in space group *P*1 were grown from 0.1 M sodium citrate (pH 4.6), 5% propanol, and 7% polyethylene glycol 4000. For data collection, crystals were transferred to a cryoprotected solution containing 15% (v/v) glycerol in the corresponding mother liquor. Diffraction data were collected at at 100 K at Beamlines X25 and X12B at the Brookhaven National Laboratory National Synchrotron Light Source. The structure of I573T/N36(L6)C34 was determined by molecular replacement using the program AmoRe.

Results: The overall architecture and helix packing of the I573T/N36(L6)C34 trimmer are the same as those of the wild-type gp41 core. Three molecules, each consisting of an N36 helix paired with an antiparallel C34 helix, pack together around the noncrystallographic three-fold axis to form a trimmer-of-hairpins. Three N36 helices form an interior, parallel coiled-coil trimmer with a left-handed superhelical pitch, while three C34 helices wrap in an oblique, antiparallel manner against the surface of the N36 coiled coil (Figure 3b). The root mean square (r.m.s.) deviation between all Cα atoms of the entire helical regions between I573T/N36(L6)C34 and the wild-type N36/C34 complex is 0.54 Å. This correspondence indicates that the Thr 573 substitution causes little distortion of the trimmer-of-hairpins structure. The Thr 573 side chains, at the ***a*** position, face each other across the molecular three-fold symmetry axis and form a network of new hydrogen bonds between the side-chain Oγ atom and the backbone carbonyl group of Thr 569 in the preceding turn of the same helix. As a consequence, a cavity (22 Å^3) is located between the Thr 569 and Thr 573 layers. No electron density was apparent in this cavity. The Thr 573 side chains move closer to each other toward the center of the coiled-coil trimmer. The radius of the coiled-coil core at this Thr 573 layer, calculated based on the average Cα-Cα distance between the residues at the same heptad layer, is 3.79 Å. By contrast, the radius of the corresponding Ile 573 layer in the wild-type N36/C34 structure is 3.94 Å. It would appear that the stabilization energy gained from the favorable hydrogen-bonding and side chain packing interactions allow the polar threonine residues to be accommodated in the interior coiled-coil core without significantly altering the trimmer-of-hairpins structure. In addition, the Cγ atoms of the Thr 573 side chains are too far from each other to make van der Waals contacts and the buried Thr 573 residue therefore contributes little to the inter-helical packing interactions.

Conclusions: This study directly demonstrates that the gp41 ectodomain core with the Ile 573 to threonine mutation is stably folded under physiological conditions, even though it is destabilized relative to the wild-type molecule. We have also shown that the fusion phenotype of the I573T envelope glycoprotein is similar to that of the wild-type envelope protein complex. Our results provide direct evidence that the stability of the trimmer-of-hairpins structure determines the membrane fusion properties of the gp120/gp41 complex. We propose that the receptor-triggered conformational changes of the HIV-1 envelope glycoprotein are thermodynamically controlled, and that the process of membrane apposition and lipid bilayer fusion is driven by the currency of energy released from the formation of the fusion-active gp41 core.

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